erage of about 5% of the absorbed C^{14} was found in the expired CO_2. Approximately 40% of the absorbed C^{14} remains to be accounted for.

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Hypoglycemic Effect of D-Ribose in Man. (23286)

STANTON SEGAL, JOSEPH FOLEY AND JAMES B. WYNGAARDEN*
(Introduced by R. W. Bates)
Natl. Inst. of Arthritis, N.I.H., U. S. Department of Health, Bethesda, Md.

Ribose 5-phosphate occupies an important position as an intermediate in the oxidative or pentose phosphate pathway of glucose metabolism. In both plant and animal tissues ribose 5-phosphate forms fructose 6-phosphate(1) which may be readily converted to glucose. Recently Agranoff and Brady(2) have isolated an enzyme from mammalian liver capable of phosphorylating D-ribose to form ribose 5-phosphate, thus permitting ribose to enter into this pathway. The conversion of ribose to glucose has been demonstrated by Katz *et al.*(3) who found that ribose 1-C^{14} incubated with rat liver slices yielded glucose labeled in a pattern which agreed with predictions from known reactions. Intravenous infusion of known glycogenic sugars such as D-galactose(4) and D-fructose(5,6) have been shown to cause an increase in blood glucose levels in normal man. Occurrence of increases in blood glucose levels after infusion of the pentoses D-xylose and L-arabinose has been reported from this laboratory although no effect was observed after D-lyxose and D-arabinose administration(7). Bruck and Rapoport(8) have reported that D-galactose infusions cause a lowering of blood glucose levels in galactosemic individuals. Except for a single observation that infusion of D-fructose may have a glucose lowering effect (9) no sugars have been observed to cause decreases in blood glucose levels following infusion into normal man. This paper reports that following infusion of D-ribose into normal man, decreases in blood glucose levels occur despite the fact that this sugar is susceptible to conversion to glucose via known metabolic pathways.

Methods. The D-ribose used was obtained from the Pfanstiehl Co., Waukegan, Ill. By the criteria of optical rotation and paper chromatography, the sugar was authentic D-ribose. Fifteen infusions of D-ribose were performed in 5 fasting normal males, (ages 18-21 yrs.) 3 diabetics (ages 23, 31 and 53 yrs.) whose last insulin dose (NPH + crystalline) was given 24 hours prior to study and one 48 yr. old subject with liver disease proved by liver biopsy. D-ribose was autoclaved as a 7.5% solution and found sterile and pyrogen-free prior to use. In 13 studies, 10 to 20 g of D-ribose were infused intravenously over 10-25 minutes. Blood specimens were obtained from an indwelling plas-

* Present address: Duke University School of Medicine, Durham, N. C.

TABLE I. Observations on Glucose and Ribose Levels in Blood after Ribose Infusion.*

Subjects	Fasting blood glucose, mg %	Min blood glucose, mg %	Time to min glucose after start of infusion, min.	Blood ribose at time of min glucose, mg %
Normals				
JD	91	59	75	7
"	63	37	56	19
LC	71	30	65	9
WY	52	39	89	10
WB	78	60	34†	54
" ‡	68	57	36†	14
WW	56	34	65	16
"	43	15	81	9
" §	61	22	60	14
" ‖	52	24	60	11
Diabetic				
MS¶	314	298	18	66
NN**	293	261	76	10
CM††	467	453	16	72
Liver disease				
WS‡	72	48	66	3

* 20 g infused except where otherwise stated.
† Exp. interrupted at times given.
‡ 10 g infused.
§ Constant infusion 97 mg/min. after 7.5 g prime. 13 g total amount.
‖ " " 136 mg/min. " 4 g " . 12 g " " .
¶ 23 yr male requiring 45 u of NPH and crystalline insulin daily.
** 31 " " " 46 u " " " " " .
†† 54 " " " 36 u of globin & " " " " .

tic catheter in the opposite arm before ribose infusion and at 5 to 10 minute intervals after the end of the infusion for a 90 minute period. Two studies were performed in one individual in whom an injection of a priming dose of ribose was given after which ribose was infused at a constant rate with the aid of a Bowman pump. Three studies were also performed in which 16 to 20 g of D-xylose were infused as a 10% solution into 3 normal males. Blood and urine glucose were determined by a method devised in this laboratory (7) similar to that of Froesch and Renold (10) employing the enzyme glucose oxidase. This method measures glucose specifically and does not include non-glucose reducing substances. Ribose was determined by the orcinol method(11) using blood filtrates in which glucose has been destroyed by glucose oxidase. Serum inorganic phosphate was measured by the method of Fiske and SubbaRow(12) and blood pyruvate by the lactic dehydrogenase method of Segal, Blair and Wyngaarden(13).

Results. Effect of infusions of D-ribose on blood glucose levels. Blood glucose levels fell in all of the normal individuals given D-ribose intravenously (Table I). The magnitude of this fall ranged from 16 to 65% of the initial level. The changes in glucose levels varied from 11 to 41 mg %. The effect of ribose could be reproduced in a given individual as shown on subjects W.W. and J.D.

The pattern of the glucose fall in normal subjects may be seen in Table II, subjects J.D. and W.W. Though slight changes in blood glucose level may be observed at the end of the infusion period (J.D.) usually 20 to 50 minutes before a phase of rapid decline of glucose levels was observed. Minimum glucose levels occurred at 56 to 89 minutes after the start of the ribose infusion (Table I) and subsequently returned to approximately fasting of values at 115 minutes. In the constant infusion experiment shown in Table II (subj. W.W.) the glucose level was depressed after 30 minutes and continued to decrease until 60 to 65 minutes when it was only 48% of the fasting value. This fall occurred during the infusion of only 136 mg of

TABLE II. Levels of Blood Glucose after Ribose and Xylose Infusion.*

Subjects	Duration of infusion, min.	Glucose (mg %) at various times (min.) after starting infusion															
		0	15	25	35	45	55	60	65	70	75	80	85	90	95	100	105	115
Ribose																		
JD	25	91		88	89	80	65		65		59	61	73	76	77	79	81	78
WW†	70	52		39		36		24	25	30								
WW‡	11	65	34	9	0	28	26	28	30	33	29	33	45	40	37			
Xylose																		
JD	13	60	73		71	62	67		68									
WR	23	74		95			96		104		98		85					
WM§	115	50	43		50		46				49							48

* 20 g infused except where otherwise stated.

† Constant infusion ribose; 136 mg/min. after 4 g prime; constant blood ribose level 11 mg %. 12 g total amount infused.

‡ 7 U crystalline insulin (0.1 U/kg) given intrav. 4 min. after start of infusion.

§ Constant infusion xylose; 66.5 mg/min. after 10 g prime; constant xylose blood level 40 mg %; 16 g total amount.

ribose/minute.

The blood glucose response in 3 subjects with diabetes mellitus and one with diffuse liver decrease is also shown in Table I. Decreases in glucose were seen in the three diabetics. However, only subject N.N. had a pattern of response similar to the normal, the others showing only a transient response at the end of the infusion period. The fall of glucose in subject W.S. with liver disease resembled that seen in the normal subject.

Relationship of blood levels of ribose to hypoglycemic response. The blood ribose concentrations present when the lowest blood glucose levels were recorded are shown in Table I. Low levels of 10-19 mg % are to be noted. Since immediately after infusion of 20 g of ribose the blood levels of ribose ranged from 60 to 80 mg %, the glucose effect cannot be correlated with a high ribose level *per se*. The fact that the time of maximal glucose fall after the single injection of 20 g corresponds with that observed during the constant infusion of small amounts of ribose (Table I subj. W.W.) indicates that the length of time during which ribose is present in the body is an important factor in the glucose responses and suggests that a metabolite rather than ribose itself may be the substance responsible for the hypoglycemic effect.

Changes of phosphate and pyruvate during ribose induced hypoglycemia. Measurement of serum inorganic phosphate and blood pyruvate levels was made in four normals whose fall ranged from 39 to 65% and one diabetic (M.S.) with a 5% glucose fall. In the normals the phosphate decreased 10 to 19% and the pyruvate changes varied from a decrease of 34% to an increase of 20%. The diabetic had a fall in phosphate and pyruvate of 19% and 13%, respectively. The decreases in phosphate levels correspond to those observed after infusion of other pentoses(7). The pyruvate changes are probably insignificant. These observations contrast with those seen after insulin induced hypoglycemia in subject W.W. in whom intravenous administration of crystalline insulin (0.1 U/kg) which produces a blood glucose fall similar to that of ribose produced a 39% fall in phosphate and an elevation of pyruvate of 300% of the control value.

Urinary glucose excretion during ribose induced hypoglycemia. Total glucose lost in urine was determined in 2 experiments (subj. J.D. and W.W.). In one, 23 mg of glucose was present in the urine collected during the period when blood glucose fell from 91 to 59 mg %. In the other the loss of glucose in the urine was 75 mg in the period when glucose fell from 43 to 15 mg %.

Effect of D-ribose upon responsiveness to insulin induced hypoglycemia. In one experiment (Table II, W.W.) 7 U (0.1 U/kg) of crystalline insulin was given intravenously through the indwelling catheter while ribose was being infused into the opposite arm and

timed so that maximal hypoglycemia due to insulin would occur about 20 minutes after the end of the ribose infusion. Whereas normally, the blood glucose levels rise to control levels about 90 minutes after insulin administration, in this experiment the blood glucose level was only 57% of the control value 93 minutes after insulin was given, thus indicating that ribose administration had prevented the usual response of blood glucose following insulin hypoglycemia.

Lack of hypoglycemic effect of D-xylose infusion. Since the pentose D-xylose also enters the pentose phosphate pathway to hexose formation(1) three studies with xylose are shown in Table II. Subject J.D.'s response to xylose differs from that to ribose. At the time when low glucose levels are seen after ribose infusion, glucose levels are elevated after xylose. Elevation of glucose levels after xylose infusion is seen also in subject W.R. In subject W.M. during the constant infusion of xylose there was no significant change in blood glucose despite administration of a quantity of xylose equal to that of ribose and the presence of a blood xylose level 4 times as high as the level of ribose at which a hypoglycemic response was noted in the constant ribose infusion study, subject W.W., Table II.

Discussion. The hypoglycemic effects of ribose on blood glucose levels of man may represent an unusual species response. After studying the metabolic effects of ribose administration to both the intact rabbit and mouse, Naito(14) reports that in these species ribose causes an increase in fermentable substance of blood as well as an increase in liver glycogen. It appears also that the effect of ribose on glucose levels in man is specific for this pentose alone and is not a general effect shared by xylose or other pentoses (7).

The mechanism of the fall in blood glucose in man resulting from infusions of D-ribose is at present obscure. It is possible that ribose causes an insulin release from the pancreas. However, the metabolic data of the present study do not support this mechanism as being responsible for the hypoglycemia resulting from infusions of ribose. Injection of insulin into man causes an increase in peripheral utilization of glucose which is accompanied by large decreases in blood inorganic phosphate (15) and an increase in blood pyruvate(16). Neither of these findings has been observed following ribose administration. Moreover, the fall in inorganic phosphate observed after ribose infusion corresponds to that observed after infusion of other pentoses which have no hypoglycemic effect(7).

In subjects J.D. and W.W. blood glucose levels decreased 32 and 28 mg % respectively. If the glucose space is 17% of the body weight(17) or 12 liters in a 70 kg man, a decrease of this magnitude of blood glucose levels corresponds to a loss of over 3 g of glucose from the glucose space. The urinary glucose loss during these experiments of 23 and 75 mg makes it apparent that the fall of glucose levels due to ribose infusion cannot be due to glucosuria.

The possibility must be considered that ribose alters liver metabolism in such a way as to interrupt the hepatic homeostatic mechanism for maintenance of blood glucose. Recently, potent hypoglycemic agents have been shown to inhibit the breakdown of glycogen in liver(18) and to lower glucose content of hepatic vein blood(19). The observation that ribose may have inhibited the normal compensatory response to insulin induced hypoglycemia suggests that this mechanism may also explain the hypoglycemia resulting from administration of ribose. Should ribose exert its effect by inhibiting hepatic glycogenolysis, a response in the diabetic subject may well depend on the presence of adequate glycogen stores.

Summary. Infusion of D-ribose into normal man causes a marked decrease in blood glucose level which is not associated with significant elevation of blood pyruvate level or large decreases in serum inorganic phosphate concentration. Though the exact mechanism of this hypoglycemia is obscure the data appear to exclude increased peripheral glucose utilization and renal glucosuria as explanations of the ribose effect.†

† Similar changes to those reported here in blood glucose after ribose infusions into normal individuals have been observed also by Dr. Howard Hiatt, Beth Israel Hospital, Boston, Mass. (personal correspondence).

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Excretion of 17-Ketosteroids in the Rhesus Monkey.* (23287)

RAYMOND VAN DE WIELE, WALTER HERRMANN, GERTRUDE VAN WAGENEN
Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Conn.

The excretion of 17-ketosteroids in the urine of the female Rhesus monkey was studied in order to determine whether this animal could be used in an experiment investigating adrenal-ovarian relationship and effect of cortisone on menstruation and 17-ketosteroid excretion. Marker isolated 4 mg of Androstanedione(1) from a 6 months pool of monkey urine. Dorfman and associates (2) reported amounts of 17-ketosteroids averaging 1.2 mg/24 hr for the female and 2.1 mg/24 hr for the male in the ketonic fraction of monkey urine, as determined by a Holtorff-Koch(3) modification of the Zimmermann reaction. Furthermore it was observed that Zimmermann chromogen was still present in the urinary extracts following removal of both gonads and adrenals. This led these investigators to the speculation that 17-ketosteroids might originate from a source other than the adrenals or the gonads.

Methods. Urine from Rhesus monkeys was collected into 2 pools over a period of several days and stored in the refrigerator. The pools measured 1080 ml (3 days) and 1500 ml (approximately 6 days) respectively. The total 17-ketosteroids were determined according to the method of Holtorff-Koch as 12 mg and 20 mg. The following procedures for fractionation and identification were then carried out on both urine pools. The urine was adjusted to pH 1.0 with 50% H_2SO_4 and extracted continuously for 48 hours with ether. The ether was washed with 5% NaOH until pigment-free, then with H_2O until neutral and the washings were added to the urine. Further hydrolysis was achieved by boiling for 30 minutes at pH 1.0. The urine was then extracted manually and washed as above. Both ether fractions were combined and dried over Na_2SO_4. After evaporation

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